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TITLE: Improved Bacillus Host Cell

TECHNICAL FIELD

Bacillus sp. are attractive hosts for the production of heterologous proteins due their ability to secrete proteins directly into the culture medium. They have a high capacity for protein secretion, are genetically highly amenable, nonpathogenic and free of endotoxins, and consequently a large variety of proteins from different organisms have been efficiently produced and secreted in *Bacillus sp. i.e.* in *Bacillus licheniformis.*

In the highly competitive biotech industry, even slightly improved *Bacillus* host cells are in demand, which may provide more attractive production systems, or may even just be alternative production systems.

BACKGROUND

Many industrial products of commercial interest can be produced biologically in *Bacillus sp.* host cells e.g. heterologous polypeptides, amino acids, carbohydrates etc. Some of these products are sold as process aids, intermediates, or even end-products in the food and feed industries as well as in the pharmaceutical industry. There are increasingly strict regulations that must be complied with when producing such products in microbial production hosts for sale in these industries, for instance traces of antibiotics in the products is seen as a problem. When producing in *Bacillus licheniformis* it is thus desirable to ensure that the host cell is not capable of producing antibiotic compounds native to the cell such as lichenysin, subtilisin, and surfactin.

SUMMARY

A problem to be solved by the present invention is how to obtain a *Bacillus licheniformis* host cell incapable of producing native antibiotic compounds, or how to impair the production of these compounds in said cell. The present invention provides a solution to the problem by providing a *Bacillus licheniformis* host cell which has a reduced capacity to produce one or more polypeptide(s) involved in antibiotic synthesis.

Accordingly, in a first aspect the invention relates to a *Bacillus licheniformis* mutant host cell derived from a parent *B. licheniformis* host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in antibiotic synthesis which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 10, preferably at least 85% identical, more preferably at least 90% identical, still more preferably at least 95% identical, and most preferably at least 97% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 10, wherein the mutant host cell expresses at least

5% less of the one or more polypeptide(s) involved in antibiotic synthesis than the parent host cell, when they are cultivated under comparable conditions.

Preferably the mutant host cell expresses at least 10% less, more preferably at least 20% less, still more preferably at least 30% less, even more preferably at least 40% less, yet more preferably at least 50% less, or at least 60% less, or at least 70% less, or at least 80%, or most preferably at least 90% less of the one or more polypeptide(s) involved in antibiotic synthesis than the parent host cell, when they are cultivated under comparable conditions. Most preferably the mutant host cell expresses absolutely nothing of the one or more polypeptide(s) involved in antibiotic synthesis.

Comparable conditions of cultivation must be used in order to compare the expression level of the one or more polypeptide(s) involved in antibiotic synthesis in a mutant host cell of the invention with that in a parent host cell. They are cultivated separately under identical conditions in identical setups, of course allowing for the usual standard deviations of the operating parameters normally associated with growth experiments, such as temperature control etc. The quantification of the expression level of the one or more polypeptide(s) is done by standard text-book assay techniques as known in the art e.g. mRNA quantification or immuno-based assays.

In a second aspect the invention relates to a process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the previous aspect in a suitable medium, whereby the said product is produced.

Finally, an aspect of the invention relates to a use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

DEFINITIONS

Nucleic acid construct: When used herein, the term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or which has been modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present invention.

Control sequence: The term "control sequences" is defined herein to include all components, which are necessary or advantageous for the expression of a polypeptide of the present invention. Each control sequence may be native or foreign to the nucleotide sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleotide sequence encoding a polypeptide.

Operably linked: The term "operably linked" is defined herein as a configuration in which a control sequence is appropriately placed at a position relative to the coding sequence of the DNA sequence such that the control sequence directs the expression of a polypeptide.

Coding sequence: When used herein the term "coding sequence" is intended to cover a nucleotide sequence, which directly specifies the amino acid sequence of its protein product. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon. The coding sequence typically include DNA, cDNA, and recombinant nucleotide sequences.

Expression: In the present context, the term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: In the present context, the term "expression vector" covers a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of the invention, and which is operably linked to additional segments that provide for its transcription.

DETAILED DISCLOSURE

A *Bacillus licheniformis* mutant host cell derived from a parent *B. licheniformis* host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in antibiotic synthesis which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 10, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in antibiotic synthesis than the parent host cell, when they are cultivated under comparable conditions.

The term "parent host cell" in the context of the present invention means a cell which is genetically identical, or isogenic, to the progeny mutant or mutant cell of the present invention, except for the mutated one or more gene(s) encoding one or more polypeptide(s) involved in antibiotic synthesis in said mutant.

The degree of identity, or %-identity of polypeptide sequences can suitably be investigated by aligning the sequences using a computer program known in the art, such as "GAP" provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711)(Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453). Using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3".

An object of the present invention is to provide a culture medium free of antibiotics so as to be able to reduce the product purification to a minimum, and to comply with regulatory requirements. This may be done according to the invention by reducing or even completely abolishing the expression of one or more gene(s) encoding a native polypeptide(s) involved in antibiotic synthesis via mutagenisation of that (those) gene(s). One of the very well-known method of ensuring that a gene is not expressed into an active polypeptide within a cell is simply to delete or partially delete the encoding gene. Many techniques have been described in the art on how to specifically delete or partially delete one or more gene(s) in the genome of a cell, and certainly from the genome of a *Bacillus licheniformis* cell (see e.g. Novozymes A/S WO 01/90393, Novozymes A/S WO 02/00907). Accordingly, a preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in antibiotic synthesis.

A preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated in two or more genes encoding two or more polypeptides involved in antibiotic synthesis.

The product of interest to be produced by the mutant host cell of the first aspect may be one or more polypeptide(s) encoded by one or more heterologous gene(s). Consequently, a preferred embodiment of the present invention relates to a host cell of the first aspect, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).

In the industrial production of polypeptides it is of interest to achieve a product yield as high as possible. One way to increase the yield is to increase the copy number of a gene encoding a polypeptide of interest. This can be done by placing the gene on a high copy number plasmid. However, plasmids are unstable and are often lost from the host cells if there is no selective pressure during the cultivation of the host cells. Another way to increase the copy number of the gene of interest is to integrate it into the host cell chromosome in multiple copies. Integration of two genes has been described in WO 91/09129 and WO 94/14968 (Novozymes A/S) the content of which is hereby incorporated by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) is present in at least two copies, preferably at least 4 copies, and most preferably at least 6 copies. In another embodiment the heterologous gene(s) is present in at least ten copies. If carried on a plasmid the gene(s) may be present in several hundred copies per cell, so in a still further embodiment of the present invention the heterologous gene(s) is present in at least 100 copies.

Integration of two genes closely spaced in anti-parallel tandem to achieve better stability has been described in WO 99/41358 (Novozymes A/S) the content of which is hereby incorporated by reference, as well as the stable chromosomal multi-copy integration of genes described in WO 02/00907 (Novozymes A/S) the content of which is incorporated herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are stably integrated into the genome of the cell.

Selection of chromosomal integrant has for convenience resulted in the use of selectable markers such as antibiotic resistance markers. However it is desirable if possible to avoid the use of antibiotic marker genes. WO 01/90393 discloses a method for the integration of a gene in the chromosome of a host cell without leaving antibiotic resistance markers behind in the strain, the content of which is hereby incorporated by reference A preferred embodiment of the present invention relates to a host cell of the first aspect wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker gene(s) at the site of integration.

The present invention also relates to nucleic acid constructs comprising a nucleotide sequence encoding a product of interest, which may be operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

A nucleotide sequence encoding a polypeptide ofinterest may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the nucleotide sequence prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying nucleotide sequences utilizing recombinant DNA methods are well known in the art.

Other ways of increasing the product yield would be to increase promoter activity of the specific promoter regulating the expression of a specific gene of interest. Also a more general increase in the activity of several promoters at the same time could lead to an improved product yield. The control sequence may be an appropriate promoter sequence, a nucleotide sequence which is recognized by a host cell for expression of the nucleotide sequence. The promoter sequence contains transcriptional control sequences, which mediate the expression of the polypeptide. The promoter may be any nucleotide sequence which shows transcriptional activity in the host cell of choice including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the E. coli lac operon, Streptomyces coelicolor agarase gene (dagA), Bacillus subtilis levansucrase gene (sacB), Bacillus licheniformis alpha-amylase gene (amyL), Bacillus stearothermophilus maltogenic amylase gene (amyM), Bacillus amyloliquefaciens alpha-amylase gene (amyQ), Bacillus licheniformis penicillinase gene (penP), Bacillus subtilis xylA and xylB genes, and prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75: 3727-3731), as well as the tac promoter (DeBoer et al., 1983, Proceedings of the National Academy of Sciences USA 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242: 74-94; and in Sambrook et al., 1989, supra.

Other useful promoters are described in WO 93/10249, WO 98/07846, and WO 99/43835 (Novozymes A/S) the contents of which are incorporated fully herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.

The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably

linked to the 3' terminus of the nucleotide sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA which is important for translation by the host cell. The leader sequence is operably linked to the 5' terminus of the nucleotide sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleotide sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the nucleotide sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to the coding sequence. The foreign signal peptide coding region may be required where the coding sequence does not naturally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the polypeptide. However, any signal peptide coding region which directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

Effective signal peptide coding regions for bacterial host cells are the signal peptide coding regions obtained from the genes for Bacillus NCIB 11837 maltogenic amylase, Bacillus stearothermophilus alpha-amylase, Bacillus licheniformis subtilisin, Bacillus licheniformis beta-lactamase, Bacillus stearothermophilus neutral proteases (nprT, nprS, nprM), and Bacillus subtilis prsA. Further signal peptides are described by Simonen and Palva, 1993, Microbiological Reviews 57: 109-137.

The control sequence may also be a propeptide coding region that codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is

known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to a mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding region may be obtained from the genes for Bacillus subtilis alkaline protease (aprE), Bacillus subtilis neutral protease (nprT), Saccharomyces cerevisiae alpha-factor, Rhizomucor miehei aspartic proteinase, and Myceliophthora thermophila laccase (WO 95/33836).

Where both signal peptide and propeptide regions are present at the amino terminus of a polypeptide, the propeptide region is positioned next to the amino terminus of a polypeptide and the signal peptide region is positioned next to the amino terminus of the propeptide region.

It may also be desirable to add regulatory sequences which allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the lac, tac, and trp operator systems. In yeast, the ADH2 system or GAL1 system may be used. In eukaryotic systems, these include the dihydrofolate reductase gene which is amplified in the presence of methotrexate, and the metallothionein genes which are amplified with heavy metals. In these cases, the nucleotide sequence encoding the polypeptide would be operably linked with the regulatory sequence.

The present invention also relates to recombinant expression vectors comprising the nucleic acid construct of the invention. The various nucleotide and control sequences described above may be joined together to produce a recombinant expression vector which may include one or more convenient restriction sites to allow for insertion or substitution of the nucleotide sequence encoding the polypeptide at such sites. Alternatively, the nucleotide sequence of the present invention may be expressed by inserting the nucleotide sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

The recombinant expression vector may be any vector (e.g., a plasmid or virus) which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

The vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome.

The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon may be used.

The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are the dal genes from Bacillus subtilis or Bacillus licheniformis, or markers which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance.

The vectors of the present invention preferably contain an element(s) that permits stable integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the nucleotide sequence encoding the polypeptide or any other element of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleotide sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleotide sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleotides, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell.

Furthermore, the integrational elements may be non-encoding or encoding nucleotide sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in E. coli, and pUB110, pE194, pTA1060, and pAMß1 permitting replication in Bacillus. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75: 1433).

More than one copy of a nucleotide sequence of the present invention may be inserted into the host cell to increase production of the gene product. An increase in the copy number of the nucleotide sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the nucleotide sequence where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the nucleotide sequence, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, e.g., Sambrook et al., 1989, supra).

The introduction of a vector into a bacterial host cell may, for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, Molecular General Genetics 168: 111-115), using competent cells (see, e.g., Young and Spizizin, 1961, Journal of Bacteriology 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, Journal of Molecular Biology 56: 209-221), electroporation (see, e.g., Shigekawa and Dower, 1988, Biotechniques 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, Journal of Bacteriology 169: 5771-5278).

A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon. The term "operon" in the context of the present invention means a polynucleotide comprising several genes that are clustered and perhaps even transcribed together into a polycistronic mRNA, e.g. genes coding for the enzymes of a metabolic pathway. The

transcription of an operon may be initiated at a promoter region and controlled by a neighboring regulatory gene, which encodes a regulatory protein, which in turn binds to the operator sequence in the operon to respectively inhibit or enhance the transcription. The gene or the operon can be carried on a suitable plasmid that can be stably maintained, e.g. capable of stable autonomous replication in the host cell (the choice of plasmid will typically depend on the compatibility of the plasmid with the host cell into which the plasmid is to be introduced) or it can be carried on the chromosome of the host. The said gene may be endogenous to the host cell in which case the product of interest is a protein naturally produced by the host cell and in most cases the gene will be in it normal position on the chromosome. If the gene encoding the product of interest is an exogenous gene, the gene could either be carried on a suitable plasmid or it could be integrated on the host chromosome. In one embodiment of the invention the eubacterium is a recombinant eubacterium. Also the product of interest may in another embodiment be a recombinant protein.

The product of interest is any gene product or product of a metabolic pathway which is industrially useful and which can be produced in a bacterial cell such as a *B. licheniformis*.

In one preferred embodiment, the heterologous polypeptide(s) is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

In another preferred embodiment, the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.

Yet another embodiment relates to a host cell of the first aspect, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants, and preferably the carbohydrates comprise hyaluronic acid.

In one embodiment the heterologous polypeptide(s) is an enzyme, particularly the enzyme is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6). Preferably the enzyme is an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase,

phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, or xylanase. Preferably the enzyme is an amylase or a mannanase.

A second aspect of the invention relates to a process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the first aspect of the invention in a suitable medium, whereby the said product is produced. One embodiment relates to a process of the second aspect, further comprising isolating or purifying the product of interest. Suitable media for the cultivation is described below as well as methods for the purification or isolation of the produced product which is an optional additional step to the process of the present invention.

In the production methods of the present invention, the cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media are prepared using procedures known in the art (see, *e.g.*, references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, *More Gene Manipulations in Fungi*, Academic Press, CA, 1991).

The polypeptides may be detected using methods known in the art that are specific for the polypeptides. These detection methods may include use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide as described herein.

The resulting polypeptide may be recovered by methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

The polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (*e.g.*, preparative isoelectric focusing), differential solubility (*e.g.*, ammonium sulfate precipitation), SDS-PAGE, or extraction (see, *e.g.*, *Protein Purification*, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

A third aspect of the present invention relates to the use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced, and optionally isolating or purifying the produced product.

CLAIMS

1. A *Bacillus licheniformis* mutant host cell derived from a parent *B. licheniformis* host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in antibiotic synthesis which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 10, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in antibiotic synthesis than the parent host cell, when they are cultivated under comparable conditions.

2. The host cell according to claim 1, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in antibiotic synthesis.

3. The host cell according to any of claims 1 – 2, which is mutated in two or more genes encoding two or more polypeptides involved in antibiotic synthesis.

5. The host cell according to any of claims 1 – 4, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).

6. The host cell according to claim 5, wherein the heterologous gene(s) is present in at least two copies.

7. The host cell according to claim 5 or 6, wherein the heterologous gene(s) are stably integrated into the genome of the cell.

8. The host cell according to any of claims 5 - 7, wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker genes at the site of integration.

9. The host cell according to any of claims 5 - 8, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.

10. The host cell according to any of claim 5 – 9, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon.

11. The host cell according to any of claims 5 – 10, wherein the heterologous polypeptide(s) is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

12. The host cell according to any of claims 5 – 10, wherein the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.

13. The host cell according to claim 12, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants.

14. The host cell according to claim 13, wherein the carbohydrates comprise hyaluronic acid.

15. The host cell according to any of claims 5 – 10, wherein the heterologous polypeptide(s) is an enzyme, preferably a secreted enzyme.

16. The host cell according to claim 15, wherein the enzyme is is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6).

17. The host cell according to claim 16, wherein the enzyme is an enzyme with an activity selected from the group of enzyme activities consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, and xylanase.

18. The host cell according to claim 17, wherein the enzyme is an amylase or a mannanase.

19. A process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in any of the claims 1 - 18 in a suitable medium, whereby the said product is produced.

20. The process according to claim 19, further comprising isolating or purifying the product of interest.

21. A use of a *Bacillus licheniformis* mutant host cell as definde in any of the claims 1 - 18 for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

22. The use according to claim 21 further comprising isolating or purifying the product of interest.

ABSTRACT

TITLE: Improved Bacillus Host Cell.

A *Bacillus licheniformis* mutant host cell derived from a parent *B. licheniformis* host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in antibiotic synthesis which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 10, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in antibiotic synthesis than the parent host cell, when they are cultivated under comparable conditions.

SEQUENCE LISTING

<110> Novozymes A/S
Jørgensen, Steen Troels
Rasmussen, Michael Dolberg
Andersen, Jens Tønne
Olesen, Peter Bjarke
Clausen, Ib Groth

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<170> PatentIn version 3.1

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cgg gca ggc gag ata ttc ggc ttc ctg ggg ccg aac ggc gcc gga aaa    629
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Thr Thr Thr Ile Arg Met Ile Val Gly His Met Ser Ile Thr Ala Gly
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cgt gac tat tta aga aag ctg acg aga gaa aag gga atg gcg gtc atc   1061
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aag gct gtg aac aaa tcg ctg gaa gac cga ttc ctg gaa atc acc gca 1397
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Asp Lys Glu Glu Ala Gln His Val
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Met Ile Val Gly His Met Ser Ile Thr Ala Gly Glu Ile Ala Val Cys
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Gly Val Ser Val Lys Glu Asn Phe Glu Lys Ala Ala Arg His Ile Gly
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Asn Leu Gln Gln Tyr Ala Arg Met Thr Lys Gly Val Thr Lys Lys Lys
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115 120 125

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130 135 140

Gln Ser Leu Leu His Asp Pro Lys Leu Leu Ile Leu Asp Glu Pro Thr
145 150 155 160

Asn Gly Leu Asp Pro Ala Gly Ile Arg Glu Ile Arg Asp Tyr Leu Arg
165 170 175

Lys Leu Thr Arg Glu Lys Gly Met Ala Val Ile Val Ser Ser His Leu
180 185 190

Leu Ser Glu Met Glu Leu Met Cys Asp Arg Ile Ala Ile Ile Gln Asn
195 200 205

Gly Lys Leu Arg Asp Ile Gln His Val His Gly Pro Ala Arg Asp Glu
210 215 220

Lys Lys Arg Tyr Tyr Ile Gln Ala Asp Asp Thr Gln Ala Leu Thr Arg
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245 250 255

Ile Glu Leu Ser Ile Gln Lys Asp Glu Val Pro Asp Leu Ile Lys His
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Val Pro Ala Gly Glu Gly Ala Met Ala Ala Ile Leu Gly Met Asp Ser
    125                 130                 135

cag gcg ctg aaa gaa gtg acg gac aaa att tcc gaa gaa gga aac ctt    965
Gln Ala Leu Lys Glu Val Thr Asp Lys Ile Ser Glu Glu Gly Asn Leu
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Tyr Ser Pro Val Arg Phe Glu Glu Thr Ile Ser Arg Leu Ile Asp Glu
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Val Arg Lys Arg Gly Glu Phe Met Asn Glu Ala Val Pro Ala Gly Glu
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Phe Glu Glu Thr Ile Ser Arg Leu Ile Asp Glu Gly Val Thr Thr Phe
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Ile Glu Ile Gly Pro Gly Lys Val Leu Ser Gly Leu Val Lys Lys Val
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80 85 90

gac att aac gaa agc aac gca gac cgg atc ggc gtc tgg gtc ggt tcc 701
Asp Ile Asn Glu Ser Asn Ala Asp Arg Ile Gly Val Trp Val Gly Ser
95 100 105

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Ile Asn Ala His Gly Thr Ser Thr Pro Tyr Asn Asp Lys Phe Glu Thr
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Pro Thr Ile Asn Ile Glu Thr Pro Asp Glu Asp Cys Asp Leu Asp Tyr
    365                 370                 375

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Lys Lys Glu Ala Arg Lys Met Asp Arg Phe Thr Gln Tyr Ala Val Val
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Ser Ala Lys Met Ala Val Glu Asp Ala Gly Leu Asp Ile Asn Glu Ser
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Asn Ala Asp Arg Ile Gly Val Trp Val Gly Ser Gly Ile Gly Gly Leu
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Glu Thr Leu Glu Gln Gln Phe Glu Thr Phe Leu Thr Lys Gly Pro Arg
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Val Ile Gln Arg Gly Asp Ala Asp Val Met Ile Ser Gly Gly Thr Glu
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Asp Ala Gly Leu Ser Pro Val Asp Ile Asp Tyr Ile Asn Ala His Gly
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<213> Bacillus licheniformis

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<222> (501)..(7655)

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atggccatca catacgcagc tgaccgttgc agtgtcacaa ttactgcgga acagcttctg    180

agccgactga aagatggcga aagccatgaa cacgcaaaag aaagctctca tgcactgaca    240

aaacagatta ttcgcgaaca ggtggcatcg cttcttcttg aatcgccgga aaagataagc    300

gacagcgaaa atttgatcta ccggggaatt gattccgtga gaatcatgag tctcgcagag    360

cgctggcgcc gagcggggcg ggaggtgtcg tttgtagagc ttgcggagga cccttcgatt    420

gaaaactggt ggagactgtt gtcctcctct aaagaagcac ctttgccaaa tgcagactat    480

caatgaagga ggtcacctaa atg cct gaa tgt caa cat aac cga aag cca tta    533
                       Met Pro Glu Cys Gln His Asn Arg Lys Pro Leu
                       1               5                  10

tca gga gcg caa gcc ggg att tgg ttt gct cag cag ctt gat ccg gaa    581
Ser Gly Ala Gln Ala Gly Ile Trp Phe Ala Gln Gln Leu Asp Pro Glu
            15                  20                  25

aat ccg atc tac aat aca gct gaa tac gtt gaa att aaa ggc ccg ctt    629
Asn Pro Ile Tyr Asn Thr Ala Glu Tyr Val Glu Ile Lys Gly Pro Leu
        30                  35                  40

gat cag gag ctt ttc gaa aaa gcg ctg cgc cat gtc att aaa gaa gct    677
Asp Gln Glu Leu Phe Glu Lys Ala Leu Arg His Val Ile Lys Glu Ala
    45                  50                  55

gaa tca ttt cat gcc aga ttt gga gaa gat caa gac gga cca tgg caa    725
Glu Ser Phe His Ala Arg Phe Gly Glu Asp Gln Asp Gly Pro Trp Gln
60                  65                  70                  75

gag atc gtt ccg tca aca gat ttt ccg cta cat tac att gat gtc agc    773
Glu Ile Val Pro Ser Thr Asp Phe Pro Leu His Tyr Ile Asp Val Ser
                80                  85                  90

tca gaa acc gat ccg gaa cag gcg gct aaa agc tgg atg atg gat gac    821
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Ser Glu Thr Asp Pro Glu Gln Ala Ala Lys Ser Trp Met Met Asp Asp
                95                  100                 105

ctt gcc cgt ccg gtt gat ctg act cgc ggc ccg ctt ttt aca gaa gcg      869
Leu Ala Arg Pro Val Asp Leu Thr Arg Gly Pro Leu Phe Thr Glu Ala
        110                 115                 120

ctt ttt aaa gcg gcg caa gat cat tac ttc tgg tat cag cga act cac      917
Leu Phe Lys Ala Ala Gln Asp His Tyr Phe Trp Tyr Gln Arg Thr His
    125                 130                 135

cat atc gca aca gac ggg ttc agc ttt aca ttg atc gcc gag cgg ctg      965
His Ile Ala Thr Asp Gly Phe Ser Phe Thr Leu Ile Ala Glu Arg Leu
140                 145                 150                 155

tca aaa ata tat acc gca ttg atg cag aac aaa tcg atc gac cag agc     1013
Ser Lys Ile Tyr Thr Ala Leu Met Gln Asn Lys Ser Ile Asp Gln Ser
                160                 165                 170

gga gcc ttt ggc tcc ctc gat ttg att ctc gcg gag gaa acg gct tac     1061
Gly Ala Phe Gly Ser Leu Asp Leu Ile Leu Ala Glu Glu Thr Ala Tyr
            175                 180                 185

cgt gca tca gaa cag tat cag gaa gat cgg cga ttt tgg ctt ggg cgt     1109
Arg Ala Ser Glu Gln Tyr Gln Glu Asp Arg Arg Phe Trp Leu Gly Arg
        190                 195                 200

ttc agc gat gaa ccg gag gtg gtc agc ctt gcc gaa agg gcg ccg cgt     1157
Phe Ser Asp Glu Pro Glu Val Val Ser Leu Ala Glu Arg Ala Pro Arg
    205                 210                 215

acc tct tca agt ttt ctt cgc agg tct gaa cac ctg cca agt gag gat     1205
Thr Ser Ser Ser Phe Leu Arg Arg Ser Glu His Leu Pro Ser Glu Asp
220                 225                 230                 235

gct gat cgt ctt ctg tct gcc gcg agc aga atg ggg gca act tgg cac     1253
Ala Asp Arg Leu Leu Ser Ala Ala Ser Arg Met Gly Ala Thr Trp His
                240                 245                 250

gaa acc gtg atg gca gca gca gca ata tat gtt cac cgt ctg aca ggg     1301
Glu Thr Val Met Ala Ala Ala Ala Ile Tyr Val His Arg Leu Thr Gly
            255                 260                 265

gca aat gac gtc gtt ctc ggg atg ccg atg atg tgc cgc ctc ggg tca     1349
Ala Asn Asp Val Val Leu Gly Met Pro Met Met Cys Arg Leu Gly Ser
        270                 275                 280

gcc gct ctt cat att ccg gga atg gtc atg aat ctc ctc ccg ctt cga     1397
Ala Ala Leu His Ile Pro Gly Met Val Met Asn Leu Leu Pro Leu Arg
    285                 290                 295

att ggc atg cag ccg caa atg agc ata gga gag cta gtc aag caa atc     1445
Ile Gly Met Gln Pro Gln Met Ser Ile Gly Glu Leu Val Lys Gln Ile
300                 305                 310                 315

tcg ggt gag atg atg aag ctg cgg cgc cat cag cat tac cgc cac gaa     1493
Ser Gly Glu Met Met Lys Leu Arg Arg His Gln His Tyr Arg His Glu
                320                 325                 330

gaa ttg cgc cgg gat ctc aag ctg ctt ggc gaa aac cag cgg ctg ttc     1541
Glu Leu Arg Arg Asp Leu Lys Leu Leu Gly Glu Asn Gln Arg Leu Phe
            335                 340                 345

ggt ccg cag ctt aat ttg atg cct ttt gag aac cgc ttg aat ttt gcc     1589
Gly Pro Gln Leu Asn Leu Met Pro Phe Glu Asn Arg Leu Asn Phe Ala
        350                 355                 360

ggc tgt caa ggc atc gtg cat aat ctt gcc acg gga cct gtg gac gat     1637
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Gly Cys Gln Gly Ile Val His Asn Leu Ala Thr Gly Pro Val Asp Asp
    365                 370                 375

ttg tca atc aac att tac ggc cgt ccg gac ggt ggc ggg cta aaa gta      1685
Leu Ser Ile Asn Ile Tyr Gly Arg Pro Asp Gly Gly Gly Leu Lys Val
380                 385                 390                 395

aac atg gat gca aac cct gct gtt tat cat gcg gat gaa ctt gaa gat      1733
Asn Met Asp Ala Asn Pro Ala Val Tyr His Ala Asp Glu Leu Glu Asp
                400                 405                 410

cac ggc aat cgt ttt ctt act ctt ctg aaa acg att gcc gtt tgc gaa      1781
His Gly Asn Arg Phe Leu Thr Leu Leu Lys Thr Ile Ala Val Cys Glu
            415                 420                 425

caa act cag ccg gtg ggg acg ttg gac att ctt ctc cct gaa gaa cgc      1829
Gln Thr Gln Pro Val Gly Thr Leu Asp Ile Leu Leu Pro Glu Glu Arg
        430                 435                 440

gct caa ata ttg att gaa tgg aat caa aca gaa cac ggg ctt ccc aaa      1877
Ala Gln Ile Leu Ile Glu Trp Asn Gln Thr Glu His Gly Leu Pro Lys
    445                 450                 455

gag agt ctt ccg cag cga ttt gaa agg atg gca aag gag tgt cct gaa      1925
Glu Ser Leu Pro Gln Arg Phe Glu Arg Met Ala Lys Glu Cys Pro Glu
460                 465                 470                 475

tct ccg gca gtc gtt tgc aat gat aag gtt ctc act tat tcc gaa tta      1973
Ser Pro Ala Val Val Cys Asn Asp Lys Val Leu Thr Tyr Ser Glu Leu
                480                 485                 490

aat caa aag gct aat caa ctg gct cac ttg ctg atc gat cag ggg gcg      2021
Asn Gln Lys Ala Asn Gln Leu Ala His Leu Leu Ile Asp Gln Gly Ala
            495                 500                 505

aaa ccc gag aca ttt att gcc ttg gcg ttg ccg cgg tca gcg gaa atg      2069
Lys Pro Glu Thr Phe Ile Ala Leu Ala Leu Pro Arg Ser Ala Glu Met
        510                 515                 520

gtt gtc agc atg ctc gcc gtt ctc aaa gct gga gcg gct tat tta cct      2117
Val Val Ser Met Leu Ala Val Leu Lys Ala Gly Ala Ala Tyr Leu Pro
    525                 530                 535

ata gat ccc gat tat ccg gct gac agg atc gag tat atg ctc aac gat      2165
Ile Asp Pro Asp Tyr Pro Ala Asp Arg Ile Glu Tyr Met Leu Asn Asp
540                 545                 550                 555

gca cag ccg ctg ctt gtt atg acc agc aag gaa gcg caa gac acg atc      2213
Ala Gln Pro Leu Leu Val Met Thr Ser Lys Glu Ala Gln Asp Thr Ile
                560                 565                 570

ggt tcg caa atg ccg aag tta atc ctc gat gaa caa act gtt atg gag      2261
Gly Ser Gln Met Pro Lys Leu Ile Leu Asp Glu Gln Thr Val Met Glu
            575                 580                 585

aaa atg tcc ggc tgt tct gaa gaa aat ccc ggc gaa cag cat tcc ggc      2309
Lys Met Ser Gly Cys Ser Glu Glu Asn Pro Gly Glu Gln His Ser Gly
        590                 595                 600

aac cag ccg gca tat atg att tat acg tcg gga tca acc ggc aga cct      2357
Asn Gln Pro Ala Tyr Met Ile Tyr Thr Ser Gly Ser Thr Gly Arg Pro
    605                 610                 615

aaa ggc gtg gtc gta caa gct gaa agc tta ttc aat ttt ttg ctg tca      2405
Lys Gly Val Val Val Gln Ala Glu Ser Leu Phe Asn Phe Leu Leu Ser
620                 625                 630                 635

atg cag gac atg ttc gcg ctt aat caa gat gac cgg ctg ctg gcc gtc      2453
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Met Gln Asp Met Phe Ala Leu Asn Gln Asp Asp Arg Leu Leu Ala Val
                640                 645                 650

act aca att gca ttc gac atc tca gca ctc gaa att tat ttg ccg ctg      2501
Thr Thr Ile Ala Phe Asp Ile Ser Ala Leu Glu Ile Tyr Leu Pro Leu
            655                 660                 665

atc agc gga tca gca gtc gtt ctt gcc gag aag gaa acg gta caa gat      2549
Ile Ser Gly Ser Ala Val Val Leu Ala Glu Lys Glu Thr Val Gln Asp
        670                 675                 680

ccg tcc gaa ttg gcc aaa atg att gaa aca tac gaa att aca ata atg      2597
Pro Ser Glu Leu Ala Lys Met Ile Glu Thr Tyr Glu Ile Thr Ile Met
    685                 690                 695

cag gct aca ccg acc ctc tgg cat gca ttg gcc tcg agc gcc ccg gaa      2645
Gln Ala Thr Pro Thr Leu Trp His Ala Leu Ala Ser Ser Ala Pro Glu
700                 705                 710                 715

aag ctc aaa ggt ctt cgt gcg ctt gtc ggc ggc gaa gct ttg caa tcc      2693
Lys Leu Lys Gly Leu Arg Ala Leu Val Gly Gly Glu Ala Leu Gln Ser
                720                 725                 730

agc ctg gcc cgg caa ttg cag cag ctc gcg tgt tct tta acg aac ctt      2741
Ser Leu Ala Arg Gln Leu Gln Gln Leu Ala Cys Ser Leu Thr Asn Leu
            735                 740                 745

tat gga ccg acg gaa aca aca att tgg tct aca gcc gct gcg ctg gaa      2789
Tyr Gly Pro Thr Glu Thr Thr Ile Trp Ser Thr Ala Ala Ala Leu Glu
        750                 755                 760

ggc aac tgt acg gaa cct ccg gga atc ggc tgt gcg att tgg aat acg      2837
Gly Asn Cys Thr Glu Pro Pro Gly Ile Gly Cys Ala Ile Trp Asn Thr
    765                 770                 775

cag ctt tat gtc ctg gac gcc gga tta cag cca gtg cct ccg gga acg      2885
Gln Leu Tyr Val Leu Asp Ala Gly Leu Gln Pro Val Pro Pro Gly Thr
780                 785                 790                 795

gct gga gaa ctt tat gtg gca gga aca ggg gta gcg cgc ggc tac ttg      2933
Ala Gly Glu Leu Tyr Val Ala Gly Thr Gly Val Ala Arg Gly Tyr Leu
                800                 805                 810

aac cga cat gct ctc acg gct gag cgc ttt att gcg aat cca tac ggg      2981
Asn Arg His Ala Leu Thr Ala Glu Arg Phe Ile Ala Asn Pro Tyr Gly
            815                 820                 825

ccg cct gga tcg cgg atg tac cgg aca ggc gac atc gtg cgc tgg cgc      3029
Pro Pro Gly Ser Arg Met Tyr Arg Thr Gly Asp Ile Val Arg Trp Arg
        830                 835                 840

gaa gac ggg tcg ctt gat tat atc ggc cgt gca gac cat caa gtc aaa      3077
Glu Asp Gly Ser Leu Asp Tyr Ile Gly Arg Ala Asp His Gln Val Lys
    845                 850                 855

att cgg ggg ttc cga att gaa atg gga gaa ata gaa gcg gtg ctt gca      3125
Ile Arg Gly Phe Arg Ile Glu Met Gly Glu Ile Glu Ala Val Leu Ala
860                 865                 870                 875

aat cat ccg gtt gtt aaa caa gct gct gct atc gtt cgt gaa gac cag      3173
Asn His Pro Val Val Lys Gln Ala Ala Ala Ile Val Arg Glu Asp Gln
                880                 885                 890

ccc ggt gac ccg cgt tta ttc gcg tat gcc gtt ccc gct tcg gga gaa      3221
Pro Gly Asp Pro Arg Leu Phe Ala Tyr Ala Val Pro Ala Ser Gly Glu
            895                 900                 905

agc ctt gat cct gcc gag ctt cgc cgc ttc gtt ggt gag aca ctg ccc      3269
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Ser Leu Asp Pro Ala Glu Leu Arg Arg Phe Val Gly Glu Thr Leu Pro
        910                 915                 920

gac tat atg att ccg tct gca ttt gtc ata ctt gat gaa ctg ccg ctt     3317
Asp Tyr Met Ile Pro Ser Ala Phe Val Ile Leu Asp Glu Leu Pro Leu
    925                 930                 935

acg ccg aac gga aag ctt gac cgc aaa tcc ctg ccg gct ccg gcg gtg     3365
Thr Pro Asn Gly Lys Leu Asp Arg Lys Ser Leu Pro Ala Pro Ala Val
940                 945                 950                 955

agt atg cat aca ggc gga cgc gag ccg agg act ccg caa gag gaa att     3413
Ser Met His Thr Gly Gly Arg Glu Pro Arg Thr Pro Gln Glu Glu Ile
                960                 965                 970

ttg tgt gat tta ttt gct gag gtg ctg ggg gtg ccg cga gtc agt att     3461
Leu Cys Asp Leu Phe Ala Glu Val Leu Gly Val Pro Arg Val Ser Ile
            975                 980                 985

gat gac agc ttt ttt gac ctc ggc gga cat tcc ctt ctg gca ggc agg     3509
Asp Asp Ser Phe Phe Asp Leu Gly Gly His Ser Leu Leu Ala Gly Arg
        990                 995                1000

ctc gtc ggc cgc att cgg gaa atg ctc ggc gtc gaa ctc gga atc         3554
Leu Val Gly Arg Ile Arg Glu Met Leu Gly Val Glu Leu Gly Ile
    1005                1010                1015

ggc cgt tta ttc gat gag ccc aca gcc gcc gga ctt gcc aaa cag         3599
Gly Arg Leu Phe Asp Glu Pro Thr Ala Ala Gly Leu Ala Lys Gln
    1020                1025                1030

ctt gat cag gcg cag agc gcc cgt ccg gca ttg cgc aaa aga gag         3644
Leu Asp Gln Ala Gln Ser Ala Arg Pro Ala Leu Arg Lys Arg Glu
    1035                1040                1045

cgc cgc aag gag att ccg ctg tcc ttc gca cag cgg cgc cta tgg         3689
Arg Arg Lys Glu Ile Pro Leu Ser Phe Ala Gln Arg Arg Leu Trp
    1050                1055                1060

ttt ttg cac tgt ttg gaa gga ccg agc ccg acc tat aat att ccg         3734
Phe Leu His Cys Leu Glu Gly Pro Ser Pro Thr Tyr Asn Ile Pro
    1065                1070                1075

gtt gtc gtt cat tta act gga gat ttg gac caa aag gcg ctg gca         3779
Val Val Val His Leu Thr Gly Asp Leu Asp Gln Lys Ala Leu Ala
    1080                1085                1090

gct gct ctg ggt gat gtg gca aca aga cat gag ccg ctt cga aca         3824
Ala Ala Leu Gly Asp Val Ala Thr Arg His Glu Pro Leu Arg Thr
    1095                1100                1105

att ttc ccg gat cag caa ggg aca aca cac cag ctg ata ttg gaa         3869
Ile Phe Pro Asp Gln Gln Gly Thr Thr His Gln Leu Ile Leu Glu
    1110                1115                1120

gag gat caa tcg cgt ccc gaa ctc act gta tcc cat gtc agc gaa         3914
Glu Asp Gln Ser Arg Pro Glu Leu Thr Val Ser His Val Ser Glu
    1125                1130                1135

cat gag ttg gaa aaa gtc ctg gct gag gcc gtc cgg cac cgt tac         3959
His Glu Leu Glu Lys Val Leu Ala Glu Ala Val Arg His Arg Tyr
    1140                1145                1150

cat tta gaa aag gag cct ccg ttt cgc gcc cag cta ttc gta ctc         4004
His Leu Glu Lys Glu Pro Pro Phe Arg Ala Gln Leu Phe Val Leu
    1155                1160                1165

gga ccg gac aaa ttt gtg ctt ctt ctg ctt ttg cac cat atg atc         4049
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Gly Pro  Asp Lys Phe Val Leu  Leu Leu Leu Leu His  His Met Ile
    1170                1175                1180

ggc gac  ggc tgg tct tta atg  ccg ctg acc cgt gat  ttg gaa acc      4094
Gly Asp  Gly Trp Ser Leu Met  Pro Leu Thr Arg Asp  Leu Glu Thr
    1185                1190                1195

gcc tat  aat gcg cgt ctg caa  ggt gag gca cct gtt  tgg gag ccg      4139
Ala Tyr  Asn Ala Arg Leu Gln  Gly Glu Ala Pro Val  Trp Glu Pro
    1200                1205                1210

ctt tct  ata caa tat gcc gac  tat gcc gta tgg cag  gaa tat ctg      4184
Leu Ser  Ile Gln Tyr Ala Asp  Tyr Ala Val Trp Gln  Glu Tyr Leu
    1215                1220                1225

ctt ggc  agt gag aac aat ccg  gac agt ctg att gcc  aaa cag ctc      4229
Leu Gly  Ser Glu Asn Asn Pro  Asp Ser Leu Ile Ala  Lys Gln Leu
    1230                1235                1240

gaa tat  tgg tcg aag gca ttg  gaa cat ctg cct gat  cag ctg gag      4274
Glu Tyr  Trp Ser Lys Ala Leu  Glu His Leu Pro Asp  Gln Leu Glu
    1245                1250                1255

ctt ccg  act gat cac ccg cgt  ccg tcg gaa tct agc  tac aga agc      4319
Leu Pro  Thr Asp His Pro Arg  Pro Ser Glu Ser Ser  Tyr Arg Ser
    1260                1265                1270

ggt acg  att gat ctc agc att  gac gaa cag ctg cac  ggc cgc ctg      4364
Gly Thr  Ile Asp Leu Ser Ile  Asp Glu Gln Leu His  Gly Arg Leu
    1275                1280                1285

ttc gat  ttg tcc cgc agc acc  gga gtc agc atg ttt  atg att ttg      4409
Phe Asp  Leu Ser Arg Ser Thr  Gly Val Ser Met Phe  Met Ile Leu
    1290                1295                1300

cag tcg  gct ctt gcc gca cta  ctg aca cgg ctc gga  gca ggc cat      4454
Gln Ser  Ala Leu Ala Ala Leu  Leu Thr Arg Leu Gly  Ala Gly His
    1305                1310                1315

gat att  ccg ctc ggc agc ccg  ata gcg gga aga aac  gat gat gct      4499
Asp Ile  Pro Leu Gly Ser Pro  Ile Ala Gly Arg Asn  Asp Asp Ala
    1320                1325                1330

tta ggc  gaa att gtc gga ttg  ttt gtt aat acg ctt  gtg ctt cga      4544
Leu Gly  Glu Ile Val Gly Leu  Phe Val Asn Thr Leu  Val Leu Arg
    1335                1340                1345

aca gat  aca tcg ggt aat ccg  agt ttt cgg gag ctt  ctg aac aga      4589
Thr Asp  Thr Ser Gly Asn Pro  Ser Phe Arg Glu Leu  Leu Asn Arg
    1350                1355                1360

gtc aga  aaa gta aac ctt gca  gcg tat gag cat cag  gac ctt ccg      4634
Val Arg  Lys Val Asn Leu Ala  Ala Tyr Glu His Gln  Asp Leu Pro
    1365                1370                1375

ttc gag  cgt ctt gtt gaa gtg  ctt aat ccg cgc agg  tca aga gcg      4679
Phe Glu  Arg Leu Val Glu Val  Leu Asn Pro Arg Arg  Ser Arg Ala
    1380                1385                1390

aga cat  ccg ctg ttc caa att  atg ctt gcc ttt caa  aat acg cct      4724
Arg His  Pro Leu Phe Gln Ile  Met Leu Ala Phe Gln  Asn Thr Pro
    1395                1400                1405

gaa cct  gag ctt gac ctt tcc  ggc ctc aag tca gac  att gaa att      4769
Glu Pro  Glu Leu Asp Leu Ser  Gly Leu Lys Ser Asp  Ile Glu Ile
    1410                1415                1420

cgc agc  gtc gga gca gcc aag  ttt gat ttg acg att  gag ctt cgc      4814
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Arg Ser Val Gly Ala Ala Lys Phe Asp Leu Thr Ile Glu Leu Arg
    1425                1430                1435

gaa cat cga aaa gcg gac gga aca ccc gca ggt atc ggc gga ttt  4859
Glu His Arg Lys Ala Asp Gly Thr Pro Ala Gly Ile Gly Gly Phe
    1440                1445                1450

ctt gaa tac agc aca gat tta ttt gag cga aat acg gtt caa aca  4904
Leu Glu Tyr Ser Thr Asp Leu Phe Glu Arg Asn Thr Val Gln Thr
    1455                1460                1465

ttg gca gaa cgg ctt gtc agg ctg ctg gat tca gca gca gac gac  4949
Leu Ala Glu Arg Leu Val Arg Leu Leu Asp Ser Ala Ala Asp Asp
    1470                1475                1480

cct gat cag ccg atc gaa aaa ctc gat att ctc ctt ccg gcc gaa  4994
Pro Asp Gln Pro Ile Glu Lys Leu Asp Ile Leu Leu Pro Ala Glu
    1485                1490                1495

cgg gaa aac atg ctg gct gat tgg agc aaa tcg tcc aac agc ata  5039
Arg Glu Asn Met Leu Ala Asp Trp Ser Lys Ser Ser Asn Ser Ile
    1500                1505                1510

cct tgt tca tct ttg ccc gtt ttg ttt gaa aag cag gcg gca aag  5084
Pro Cys Ser Ser Leu Pro Val Leu Phe Glu Lys Gln Ala Ala Lys
    1515                1520                1525

gat ccg gaa gct gtt gcg gtt ata tgt gaa aat aat gcg ctc aca  5129
Asp Pro Glu Ala Val Ala Val Ile Cys Glu Asn Asn Ala Leu Thr
    1530                1535                1540

tac ggc gag ctt aat aaa cgc gct aac cgg ctt gct cat ctc ctg  5174
Tyr Gly Glu Leu Asn Lys Arg Ala Asn Arg Leu Ala His Leu Leu
    1545                1550                1555

atc gcc aaa ggg gtc ggc ccc gaa caa ttt gct gcc ctg gcg ctg  5219
Ile Ala Lys Gly Val Gly Pro Glu Gln Phe Ala Ala Leu Ala Leu
    1560                1565                1570

cca aga tct ttg gat atg gtt gtc gga ttg ctt gca gtg ctg aag  5264
Pro Arg Ser Leu Asp Met Val Val Gly Leu Leu Ala Val Leu Lys
    1575                1580                1585

gct ggc gcg gca tat gtg ccc cta gat ccg gac tac ccg gcg gaa  5309
Ala Gly Ala Ala Tyr Val Pro Leu Asp Pro Asp Tyr Pro Ala Glu
    1590                1595                1600

agg ata gcg ttt atg ttg aat gat gcc cat cct gtc tgc atc gta  5354
Arg Ile Ala Phe Met Leu Asn Asp Ala His Pro Val Cys Ile Val
    1605                1610                1615

acg agt tca gcg gta gaa tca aat ctt tct gtt ccc gga tct gtt  5399
Thr Ser Ser Ala Val Glu Ser Asn Leu Ser Val Pro Gly Ser Val
    1620                1625                1630

gaa agg att gta ctc gat gat cct tgt ata caa gag gaa ctg aaa  5444
Glu Arg Ile Val Leu Asp Asp Pro Cys Ile Gln Glu Glu Leu Lys
    1635                1640                1645

gga tgc gct gct gcg aat ccg tgt gat gcc gac cgc acc gcg cct  5489
Gly Cys Ala Ala Ala Asn Pro Cys Asp Ala Asp Arg Thr Ala Pro
    1650                1655                1660

ttg ctg cct ctt cac ccg gcg tat gtc att tac aca tcc ggt tcg  5534
Leu Leu Pro Leu His Pro Ala Tyr Val Ile Tyr Thr Ser Gly Ser
    1665                1670                1675

acc ggc aag ccg aaa gga gtc gtt gtt ccg cat caa aat gtc gtg  5579
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Thr Gly  Lys Pro Lys Gly Val  Val Val Pro His  Gln  Asn Val Val
    1680                 1685                 1690

cgg ctg  ttc gga gcg act gac  caa tgg ttc cat  ttc  ggc gct gac      5624
Arg Leu  Phe Gly Ala Thr Asp  Gln Trp Phe His  Phe  Gly Ala Asp
    1695                 1700                 1705

gat gtt  tgg acg atg ttt cat  tct tac gca ttt  gac  ttt tcg gta      5669
Asp Val  Trp Thr Met Phe His  Ser Tyr Ala Phe  Asp  Phe Ser Val
    1710                 1715                 1720

tgg gaa  att tgg gga gca ctt  ctc aat ggg ggc  cgg  ctt atc gtg      5714
Trp Glu  Ile Trp Gly Ala Leu  Leu Asn Gly Gly  Arg  Leu Ile Val
    1725                 1730                 1735

gtg cct  cat aca atc agc cgc  tcg ccg gcc gaa  ttt  ctc aat ctt      5759
Val Pro  His Thr Ile Ser Arg  Ser Pro Ala Glu  Phe  Leu Asn Leu
    1740                 1745                 1750

ctc gtt  cgc gag gga gtg act  gtg ctc aat cag  act  cct tca gca      5804
Leu Val  Arg Glu Gly Val Thr  Val Leu Asn Gln  Thr  Pro Ser Ala
    1755                 1760                 1765

ttt tat  caa ttg atg cag gct  gac cgg gac aat  gct  gag aca gga      5849
Phe Tyr  Gln Leu Met Gln Ala  Asp Arg Asp Asn  Ala  Glu Thr Gly
    1770                 1775                 1780

aag ctc  ctt tcc ttg cgc ttt  atc att ttt gga  ggg  gaa gcg ctc      5894
Lys Leu  Leu Ser Leu Arg Phe  Ile Ile Phe Gly  Gly  Glu Ala Leu
    1785                 1790                 1795

gag ctg  aag agg ctt gaa gat  tgg tat gaa cgc  cat  ccg gat cat      5939
Glu Leu  Lys Arg Leu Glu Asp  Trp Tyr Glu Arg  His  Pro Asp His
    1800                 1805                 1810

ttc ccg  cgg ctg att aat atg  tat gga att aca  gaa  acg acg gtg      5984
Phe Pro  Arg Leu Ile Asn Met  Tyr Gly Ile Thr  Glu  Thr Thr Val
    1815                 1820                 1825

cat gtc  agc tat att tct ctc  gat cag cag act  gcg  gca ctt caa      6029
His Val  Ser Tyr Ile Ser Leu  Asp Gln Gln Thr  Ala  Ala Leu Gln
    1830                 1835                 1840

gcg aac  agc ctg atc ggc cag  ggg att ccc gat  tta  ggt gta tat      6074
Ala Asn  Ser Leu Ile Gly Gln  Gly Ile Pro Asp  Leu  Gly Val Tyr
    1845                 1850                 1855

gtg ctt  gat gag tat ctt gaa  ccg gtg ccc ccg  ggc  gtc acc gga      6119
Val Leu  Asp Glu Tyr Leu Glu  Pro Val Pro Pro  Gly  Val Thr Gly
    1860                 1865                 1870

gag atg  tat gtg tcc ggg ggc  ggt ctc gca cgg  ggt  tac ttg ggc      6164
Glu Met  Tyr Val Ser Gly Gly  Gly Leu Ala Arg  Gly  Tyr Leu Gly
    1875                 1880                 1885

aga ccc  gat ttg aca gca gac  cgc ttt gtc gct  gat  ccg ttt ggt      6209
Arg Pro  Asp Leu Thr Ala Asp  Arg Phe Val Ala  Asp  Pro Phe Gly
    1890                 1895                 1900

ccg ccg  gga acc cgg atg tac  cgc acc ggc gat  ctt  gcc cgc agg      6254
Pro Pro  Gly Thr Arg Met Tyr  Arg Thr Gly Asp  Leu  Ala Arg Arg
    1905                 1910                 1915

cgc cag  gac ggc tct ctg gat  tat atg ggg cgg  gcc  gac cag cag      6299
Arg Gln  Asp Gly Ser Leu Asp  Tyr Met Gly Arg  Ala  Asp Gln Gln
    1920                 1925                 1930

atc aag  atc cgc ggc ttc aga  atc gaa ctc ggc  gag  ata gaa gcg      6344
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Ile Lys  Ile Arg Gly Phe Arg  Ile Glu Leu Gly Glu  Ile Glu Ala
    1935                 1940                 1945

gtg ctt  gtc cgc cat cat aga  gtg aat caa gcc gct  gtg gtc gta      6389
Val Leu  Val Arg His His Arg  Val Asn Gln Ala Ala  Val Val Val
    1950                 1955                 1960

agg gaa  ggc cag ccc ggg gac  aag cgt ctg atc gcc  tac gtt gtc      6434
Arg Glu  Gly Gln Pro Gly Asp  Lys Arg Leu Ile Ala  Tyr Val Val
    1965                 1970                 1975

ccg gcg  tct gaa gaa gaa acc  gat ccg gct gag ctg  cga cgg ttt      6479
Pro Ala  Ser Glu Glu Glu Thr  Asp Pro Ala Glu Leu  Arg Arg Phe
    1980                 1985                 1990

gcc gca  ggc act ctg ccg gag  tac atg gtg cct tcc  gca ttt gta      6524
Ala Ala  Gly Thr Leu Pro Glu  Tyr Met Val Pro Ser  Ala Phe Val
    1995                 2000                 2005

aaa att  tca gag ctg cca ttg  act cca aat ggc aaa  ctt gac cga      6569
Lys Ile  Ser Glu Leu Pro Leu  Thr Pro Asn Gly Lys  Leu Asp Arg
    2010                 2015                 2020

aaa gcg  ctg cct gag cct gac  ttt gcc gcg gct gtt  aaa ggg cgg      6614
Lys Ala  Leu Pro Glu Pro Asp  Phe Ala Ala Ala Val  Lys Gly Arg
    2025                 2030                 2035

ggc cca  aga aca ccg cag gaa  gaa atc ctg tgc gat  ctt ttt tca      6659
Gly Pro  Arg Thr Pro Gln Glu  Glu Ile Leu Cys Asp  Leu Phe Ser
    2040                 2045                 2050

gaa atc  ctc aat gcg ccg aga  gtc ggt att gat gac  gga ttc ttc      6704
Glu Ile  Leu Asn Ala Pro Arg  Val Gly Ile Asp Asp  Gly Phe Phe
    2055                 2060                 2065

gaa ctt  ggc ggg cat tct ctt  ctc gcc gta cag ctg  atg agc cgc      6749
Glu Leu  Gly Gly His Ser Leu  Leu Ala Val Gln Leu  Met Ser Arg
    2070                 2075                 2080

att cgt  gaa gcg ctc gga gtc  gaa ctc ggc atc ggc  gac ctg ctt      6794
Ile Arg  Glu Ala Leu Gly Val  Glu Leu Gly Ile Gly  Asp Leu Leu
    2085                 2090                 2095

gaa gcg  cct aca gtc agc gga  ctt gct gaa cgg ctt  gaa tcc ggc      6839
Glu Ala  Pro Thr Val Ser Gly  Leu Ala Glu Arg Leu  Glu Ser Gly
    2100                 2105                 2110

ggc agg  caa agt gcg ctg gat  gta atg ctt ccg ctg  aga acc gga      6884
Gly Arg  Gln Ser Ala Leu Asp  Val Met Leu Pro Leu  Arg Thr Gly
    2115                 2120                 2125

ggc agt  caa gat ccc ctg ttc  tgt gtg cac ccg gcc  gga ggc ctc      6929
Gly Ser  Gln Asp Pro Leu Phe  Cys Val His Pro Ala  Gly Gly Leu
    2130                 2135                 2140

agc tgg  tgt tat gcc ggt tta  atg acg gca ctc ggc  aag gaa tac      6974
Ser Trp  Cys Tyr Ala Gly Leu  Met Thr Ala Leu Gly  Lys Glu Tyr
    2145                 2150                 2155

ccg ata  tac ggc ctt caa gcc  cgc ggg atc gca agg  cag gaa gag      7019
Pro Ile  Tyr Gly Leu Gln Ala  Arg Gly Ile Ala Arg  Gln Glu Glu
    2160                 2165                 2170

cta cct  gat acg ctc gac gac  atg gct gct gat tat  att cgg cac      7064
Leu Pro  Asp Thr Leu Asp Asp  Met Ala Ala Asp Tyr  Ile Arg His
    2175                 2180                 2185

att cgt  acg att cag cca aca  ggc cct tat cgt ctg  ctc ggc tgg      7109
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Ile Arg  Thr Ile Gln Pro Thr  Gly Pro Tyr Arg Leu  Leu Gly Trp
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tcc ctc  gga ggc aat gtt gtt  cat gcg atc gca act  cag ctg cag      7154
Ser Leu  Gly Gly Asn Val Val  His Ala Ile Ala Thr  Gln Leu Gln
    2205                2210                2215

gaa caa  ggg gaa gac ata tcg  ctt ctt gtc atg ctt  gat gct tac      7199
Glu Gln  Gly Glu Asp Ile Ser  Leu Leu Val Met Leu  Asp Ala Tyr
    2220                2225                2230

ccg aat  cac ttc ctt cca att  aaa gat gcg cct gat  gag cag gaa      7244
Pro Asn  His Phe Leu Pro Ile  Lys Asp Ala Pro Asp  Glu Gln Glu
    2235                2240                2245

gca ttg  att gcc ctc ttg gca  ttg gga gga tac gac  ccg gac agc      7289
Ala Leu  Ile Ala Leu Leu Ala  Leu Gly Gly Tyr Asp  Pro Asp Ser
    2250                2255                2260

ctt gac  ggg gca ccg ctg aac  ctt tcc agt gcg atc  gat att ctg      7334
Leu Asp  Gly Ala Pro Leu Asn  Leu Ser Ser Ala Ile  Asp Ile Leu
    2265                2270                2275

cgc cgc  gac ggc agt gcg ctt  gcc agc ctg gac gag  gct gcg atc      7379
Arg Arg  Asp Gly Ser Ala Leu  Ala Ser Leu Asp Glu  Ala Ala Ile
    2280                2285                2290

ttg aac  tta aag gag acg tat  gtg aat tcc gtc cgg  att tta agc      7424
Leu Asn  Leu Lys Glu Thr Tyr  Val Asn Ser Val Arg  Ile Leu Ser
    2295                2300                2305

gaa tat  aag ccc cgc gtc ttt  cat ggc gat att ctg  ttt ttc aaa      7469
Glu Tyr  Lys Pro Arg Val Phe  His Gly Asp Ile Leu  Phe Phe Lys
    2310                2315                2320

tca acc  gtg ata ccg gaa tgg  ttt gat ccg atc gat  ccg gag tca      7514
Ser Thr  Val Ile Pro Glu Trp  Phe Asp Pro Ile Asp  Pro Glu Ser
    2325                2330                2335

tgg cta  cct tat ttg aac gga  aac att gat att cat  gat atg gat      7559
Trp Leu  Pro Tyr Leu Asn Gly  Asn Ile Asp Ile His  Asp Met Asp
    2340                2345                2350

tgc cgg  cat aaa gat ttg tgc  cag ccg gag ccg ctg  gcc gaa atc      7604
Cys Arg  His Lys Asp Leu Cys  Gln Pro Glu Pro Leu  Ala Glu Ile
    2355                2360                2365

gga cgt  cgg gtt tcg gaa aag  ctg gac gac ttg aaa  aaa gat acg      7649
Gly Arg  Arg Val Ser Glu Lys  Leu Asp Asp Leu Lys  Lys Asp Thr
    2370                2375                2380

gat aag  tagggagaga tagcgatgac aaatcctttt gaaaatgaac acggcacata    7705
Asp Lys
    2385

tgtcgtttta atgaatcagg atggccagta ttcactctgg ccggccttca ttcatattcc  7765

ggcagggtgg gaggtcgtct gcggggaagc gagccgcagc gcctgtatcg actatatcag  7825

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Thr Ala Glu Tyr Val Glu Ile Lys Gly Pro Leu Asp Gln Glu Leu Phe
35 40 45

Glu Lys Ala Leu Arg His Val Ile Lys Glu Ala Glu Ser Phe His Ala
50 55 60

Arg Phe Gly Glu Asp Gln Asp Gly Pro Trp Gln Glu Ile Val Pro Ser
65 70 75 80

Thr Asp Phe Pro Leu His Tyr Ile Asp Val Ser Ser Glu Thr Asp Pro
85 90 95

Glu Gln Ala Ala Lys Ser Trp Met Met Asp Asp Leu Ala Arg Pro Val
100 105 110

Asp Leu Thr Arg Gly Pro Leu Phe Thr Glu Ala Leu Phe Lys Ala Ala
115 120 125

Gln Asp His Tyr Phe Trp Tyr Gln Arg Thr His His Ile Ala Thr Asp
130 135 140

Gly Phe Ser Phe Thr Leu Ile Ala Glu Arg Leu Ser Lys Ile Tyr Thr
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Ala Leu Met Gln Asn Lys Ser Ile Asp Gln Ser Gly Ala Phe Gly Ser
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Leu Asp Leu Ile Leu Ala Glu Glu Thr Ala Tyr Arg Ala Ser Glu Gln
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Tyr Gln Glu Asp Arg Arg Phe Trp Leu Gly Arg Phe Ser Asp Glu Pro
195 200 205

Glu Val Val Ser Leu Ala Glu Arg Ala Pro Arg Thr Ser Ser Ser Phe
210 215 220

Leu Arg Arg Ser Glu His Leu Pro Ser Glu Asp Ala Asp Arg Leu Leu
225 230 235 240

Ser Ala Ala Ser Arg Met Gly Ala Thr Trp His Glu Thr Val Met Ala
245 250 255

Ala Ala Ala Ile Tyr Val His Arg Leu Thr Gly Ala Asn Asp Val Val
260 265 270

Leu Gly Met Pro Met Met Cys Arg Leu Gly Ser Ala Ala Leu His Ile
275 280 285

Pro Gly Met Val Met Asn Leu Leu Pro Leu Arg Ile Gly Met Gln Pro
290 295 300

Gln Met Ser Ile Gly Glu Leu Val Lys Gln Ile Ser Gly Glu Met Met
305 310 315 320

Lys Leu Arg Arg His Gln His Tyr Arg His Glu Glu Leu Arg Arg Asp
325 330 335

Leu Lys Leu Leu Gly Glu Asn Gln Arg Leu Phe Gly Pro Gln Leu Asn
340 345 350

Leu Met Pro Phe Glu Asn Arg Leu Asn Phe Ala Gly Cys Gln Gly Ile
355 360 365

Val His Asn Leu Ala Thr Gly Pro Val Asp Asp Leu Ser Ile Asn Ile
370 375 380

Tyr Gly Arg Pro Asp Gly Gly Gly Leu Lys Val Asn Met Asp Ala Asn
385 390 395 400

Pro Ala Val Tyr His Ala Asp Glu Leu Glu Asp His Gly Asn Arg Phe
405 410 415

Leu Thr Leu Leu Lys Thr Ile Ala Val Cys Glu Gln Thr Gln Pro Val
420 425 430

Gly Thr Leu Asp Ile Leu Leu Pro Glu Glu Arg Ala Gln Ile Leu Ile
435 440 445

Glu Trp Asn Gln Thr Glu His Gly Leu Pro Lys Glu Ser Leu Pro Gln
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Arg Phe Glu Arg Met Ala Lys Glu Cys Pro Glu Ser Pro Ala Val Val
465 470 475 480

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Cys Asn Asp Lys Val Leu Thr Tyr Ser Glu Leu Asn Gln Lys Ala Asn
                485                 490                 495

Gln Leu Ala His Leu Leu Ile Asp Gln Gly Ala Lys Pro Glu Thr Phe
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Ile Ala Leu Ala Leu Pro Arg Ser Ala Glu Met Val Val Ser Met Leu
        515                 520                 525

Ala Val Leu Lys Ala Gly Ala Ala Tyr Leu Pro Ile Asp Pro Asp Tyr
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Pro Ala Asp Arg Ile Glu Tyr Met Leu Asn Asp Ala Gln Pro Leu Leu
545                 550                 555                 560

Val Met Thr Ser Lys Glu Ala Gln Asp Thr Ile Gly Ser Gln Met Pro
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Lys Leu Ile Leu Asp Glu Gln Thr Val Met Glu Lys Met Ser Gly Cys
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Ser Glu Glu Asn Pro Gly Glu Gln His Ser Gly Asn Gln Pro Ala Tyr
        595                 600                 605

Met Ile Tyr Thr Ser Gly Ser Thr Gly Arg Pro Lys Gly Val Val Val
    610                 615                 620

Gln Ala Glu Ser Leu Phe Asn Phe Leu Leu Ser Met Gln Asp Met Phe
625                 630                 635                 640

Ala Leu Asn Gln Asp Asp Arg Leu Leu Ala Val Thr Thr Ile Ala Phe
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Asp Ile Ser Ala Leu Glu Ile Tyr Leu Pro Leu Ile Ser Gly Ser Ala
            660                 665                 670

Val Val Leu Ala Glu Lys Glu Thr Val Gln Asp Pro Ser Glu Leu Ala
        675                 680                 685

Lys Met Ile Glu Thr Tyr Glu Ile Thr Ile Met Gln Ala Thr Pro Thr
    690                 695                 700

Leu Trp His Ala Leu Ala Ser Ser Ala Pro Glu Lys Leu Lys Gly Leu
705                 710                 715                 720

Arg Ala Leu Val Gly Gly Glu Ala Leu Gln Ser Ser Leu Ala Arg Gln
                725                 730                 735

Leu Gln Gln Leu Ala Cys Ser Leu Thr Asn Leu Tyr Gly Pro Thr Glu
            740                 745                 750
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Thr Thr Ile Trp Ser Thr Ala Ala Ala Leu Glu Gly Asn Cys Thr Glu
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Pro Pro Gly Ile Gly Cys Ala Ile Trp Asn Thr Gln Leu Tyr Val Leu
    770                 775                 780

Asp Ala Gly Leu Gln Pro Val Pro Pro Gly Thr Ala Gly Glu Leu Tyr
785                 790                 795                 800

Val Ala Gly Thr Gly Val Ala Arg Gly Tyr Leu Asn Arg His Ala Leu
                805                 810                 815

Thr Ala Glu Arg Phe Ile Ala Asn Pro Tyr Gly Pro Pro Gly Ser Arg
            820                 825                 830

Met Tyr Arg Thr Gly Asp Ile Val Arg Trp Arg Glu Asp Gly Ser Leu
        835                 840                 845

Asp Tyr Ile Gly Arg Ala Asp His Gln Val Lys Ile Arg Gly Phe Arg
    850                 855                 860

Ile Glu Met Gly Glu Ile Glu Ala Val Leu Ala Asn His Pro Val Val
865                 870                 875                 880

Lys Gln Ala Ala Ala Ile Val Arg Glu Asp Gln Pro Gly Asp Pro Arg
                885                 890                 895

Leu Phe Ala Tyr Ala Val Pro Ala Ser Gly Glu Ser Leu Asp Pro Ala
            900                 905                 910

Glu Leu Arg Arg Phe Val Gly Glu Thr Leu Pro Asp Tyr Met Ile Pro
        915                 920                 925

Ser Ala Phe Val Ile Leu Asp Glu Leu Pro Leu Thr Pro Asn Gly Lys
    930                 935                 940

Leu Asp Arg Lys Ser Leu Pro Ala Pro Ala Val Ser Met His Thr Gly
945                 950                 955                 960

Gly Arg Glu Pro Arg Thr Pro Gln Glu Glu Ile Leu Cys Asp Leu Phe
                965                 970                 975

Ala Glu Val Leu Gly Val Pro Arg Val Ser Ile Asp Asp Ser Phe Phe
            980                 985                 990

Asp Leu Gly Gly His Ser Leu Leu Ala Gly Arg Leu Val Gly Arg Ile
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Arg Glu Met Leu Gly Val Glu Leu Gly Ile Gly Arg Leu Phe Asp
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Ser Ala Arg Pro Ala Leu Arg Lys Arg Glu Arg Arg Lys Glu Ile
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Pro Leu Ser Phe Ala Gln Arg Arg Leu Trp Phe Leu His Cys Leu
1055 1060 1065

Glu Gly Pro Ser Pro Thr Tyr Asn Ile Pro Val Val Val His Leu
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Thr Gly Asp Leu Asp Gln Lys Ala Leu Ala Ala Ala Leu Gly Asp
1085 1090 1095

Val Ala Thr Arg His Glu Pro Leu Arg Thr Ile Phe Pro Asp Gln
1100 1105 1110

Gln Gly Thr Thr His Gln Leu Ile Leu Glu Glu Asp Gln Ser Arg
1115 1120 1125

Pro Glu Leu Thr Val Ser His Val Ser Glu His Glu Leu Glu Lys
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Val Leu Ala Glu Ala Val Arg His Arg Tyr His Leu Glu Lys Glu
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Pro Pro Phe Arg Ala Gln Leu Phe Val Leu Gly Pro Asp Lys Phe
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Val Leu Leu Leu Leu Leu His His Met Ile Gly Asp Gly Trp Ser
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Leu Met Pro Leu Thr Arg Asp Leu Glu Thr Ala Tyr Asn Ala Arg
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Leu Gln Gly Glu Ala Pro Val Trp Glu Pro Leu Ser Ile Gln Tyr
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Ala Asp Tyr Ala Val Trp Gln Glu Tyr Leu Leu Gly Ser Glu Asn
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Asn Pro Asp Ser Leu Ile Ala Lys Gln Leu Glu Tyr Trp Ser Lys
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Ala Leu Glu His Leu Pro Asp Gln Leu Glu Leu Pro Thr Asp His
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Pro Arg Pro Ser Glu Ser Ser Tyr Arg Ser Gly Thr Ile Asp Leu
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Ser Ile Asp Glu Gln Leu His Gly Arg Leu Phe Asp Leu Ser Arg
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Ser Thr Gly Val Ser Met Phe Met Ile Leu Gln Ser Ala Leu Ala
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Ala Leu Leu Thr Arg Leu Gly Ala Gly His Asp Ile Pro Leu Gly
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Ser Pro Ile Ala Gly Arg Asn Asp Asp Ala Leu Gly Glu Ile Val
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Gly Leu Phe Val Asn Thr Leu Val Leu Arg Thr Asp Thr Ser Gly
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Asn Pro Ser Phe Arg Glu Leu Leu Asn Arg Val Arg Lys Val Asn
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Leu Ala Ala Tyr Glu His Gln Asp Leu Pro Phe Glu Arg Leu Val
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Ala Lys Phe Asp Leu Thr Ile Glu Leu Arg Glu His Arg Lys Ala
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Glu Lys Leu Asp Ile Leu Leu Pro Ala Glu Arg Glu Asn Met Leu
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Met Val Val Gly Leu Leu Ala Val Leu Lys Ala Gly Ala Ala Tyr
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Pro Ala Tyr Val Ile Tyr Thr Ser Gly Ser Thr Gly Lys Pro Lys
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Phe His Ser Tyr Ala Phe Asp Phe Ser Val Trp Glu Ile Trp Gly
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1775 1780 1785

Arg Phe Ile Ile Phe Gly Gly Glu Ala Leu Glu Leu Lys Arg Leu
1790 1795 1800

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1805 1810 1815

Asn Met Tyr Gly Ile Thr Glu Thr Thr Val His Val Ser Tyr Ile
1820 1825 1830

Ser Leu Asp Gln Gln Thr Ala Ala Leu Gln Ala Asn Ser Leu Ile
1835 1840 1845

Gly Gln Gly Ile Pro Asp Leu Gly Val Tyr Val Leu Asp Glu Tyr
1850 1855 1860

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1865 1870 1875

Gly Gly Gly Leu Ala Arg Gly Tyr Leu Gly Arg Pro Asp Leu Thr
1880 1885 1890

Ala Asp Arg Phe Val Ala Asp Pro Phe Gly Pro Pro Gly Thr Arg
1895 1900 1905

Met Tyr Arg Thr Gly Asp Leu Ala Arg Arg Arg Gln Asp Gly Ser
1910 1915 1920

Leu Asp Tyr Met Gly Arg Ala Asp Gln Gln Ile Lys Ile Arg Gly
1925 1930 1935

Phe Arg Ile Glu Leu Gly Glu Ile Glu Ala Val Leu Val Arg His
1940 1945 1950

His Arg Val Asn Gln Ala Ala Val Val Val Arg Glu Gly Gln Pro
1955 1960 1965

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1970 1975 1980

Glu Thr Asp Pro Ala Glu Leu Arg Arg Phe Ala Ala Gly Thr Leu
1985 1990 1995

Pro Glu Tyr Met Val Pro Ser Ala Phe Val Lys Ile Ser Glu Leu
2000 2005 2010

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2015 2020 2025

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Gly Val Glu Leu Gly Ile Gly Asp Leu Leu Glu Ala Pro Thr Val
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Gly Lys Val Ile Val Val Thr Ile Asn Tyr Arg Leu Gly Pro Phe Gly
30 35 40

ttt ttg cat cta tcc tca att gat gat tcc tac agc agc aat ctt ggc 497

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Phe Leu His Leu Ser Ser Ile Asp Asp Ser Tyr Ser Ser Asn Leu Gly
    45                  50                  55

ctg ctg gat caa atc gcg gct ctc gag tgg gtg aaa gac aat atc gct      545
Leu Leu Asp Gln Ile Ala Ala Leu Glu Trp Val Lys Asp Asn Ile Ala
60                  65                  70                  75

ttc ttt ggc gga gac cgt cat cac att acg gtt ttt gga gag tcg gcg      593
Phe Phe Gly Gly Asp Arg His His Ile Thr Val Phe Gly Glu Ser Ala
                80                  85                  90

gga tcg atg agc atc gct tcg ctt ttg gcg atg ccg aaa gca aag ggg      641
Gly Ser Met Ser Ile Ala Ser Leu Leu Ala Met Pro Lys Ala Lys Gly
            95                  100                 105

ctt ttt caa cag gcc att atg gaa agc ggg gct tcc gca act atg tcc      689
Leu Phe Gln Gln Ala Ile Met Glu Ser Gly Ala Ser Ala Thr Met Ser
        110                 115                 120

gat aag ctt gcg aaa gct gca gca gaa aga ttc tta agg att ctc gat      737
Asp Lys Leu Ala Lys Ala Ala Ala Glu Arg Phe Leu Arg Ile Leu Asp
    125                 130                 135

att gat cat cat cat ctg gag cgc ctt cat gat gta tct gat caa gaa      785
Ile Asp His His His Leu Glu Arg Leu His Asp Val Ser Asp Gln Glu
140                 145                 150                 155

ctt ctt gaa gcc gcc gat cag ctg cgc act tta atg gga gaa aat att      833
Leu Leu Glu Ala Ala Asp Gln Leu Arg Thr Leu Met Gly Glu Asn Ile
                160                 165                 170

ttt gaa ttg att ttt ctg cct gcg ctt gac gaa aaa acc ttg ccg ctg      881
Phe Glu Leu Ile Phe Leu Pro Ala Leu Asp Glu Lys Thr Leu Pro Leu
            175                 180                 185

aag ccg gag gtc gcc gtc gca aaa ggc gcg gca aaa gag atc aat cta      929
Lys Pro Glu Val Ala Val Ala Lys Gly Ala Ala Lys Glu Ile Asn Leu
        190                 195                 200

tta atc gga acc aaa ccc gtg atg aag gcg tct gtt ttt tcc tct gat      977
Leu Ile Gly Thr Lys Pro Val Met Lys Ala Ser Val Phe Ser Ser Asp
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tcg aaa ctt gct gag agc aat ag                                      1000
Ser Lys Leu Ala Glu Ser Asn
220                 225
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<211> 226

<212> PRT

<213> Bacillus licheniformis

<400> 10

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Pro Leu Tyr Asp Gly Thr Gln Leu Ala Lys Gln Gly Lys Val Ile Val
            20                  25                  30
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Val Thr Ile Asn Tyr Arg Leu Gly Pro Phe Gly Phe Leu His Leu Ser
35 40 45

Ser Ile Asp Asp Ser Tyr Ser Ser Asn Leu Gly Leu Leu Asp Gln Ile
50 55 60

Ala Ala Leu Glu Trp Val Lys Asp Asn Ile Ala Phe Phe Gly Gly Asp
65 70 75 80

Arg His His Ile Thr Val Phe Gly Glu Ser Ala Gly Ser Met Ser Ile
85 90 95

Ala Ser Leu Leu Ala Met Pro Lys Ala Lys Gly Leu Phe Gln Gln Ala
100 105 110

Ile Met Glu Ser Gly Ala Ser Ala Thr Met Ser Asp Lys Leu Ala Lys
115 120 125

Ala Ala Ala Glu Arg Phe Leu Arg Ile Leu Asp Ile Asp His His His
130 135 140

Leu Glu Arg Leu His Asp Val Ser Asp Gln Glu Leu Leu Glu Ala Ala
145 150 155 160

Asp Gln Leu Arg Thr Leu Met Gly Glu Asn Ile Phe Glu Leu Ile Phe
165 170 175

Leu Pro Ala Leu Asp Glu Lys Thr Leu Pro Leu Lys Pro Glu Val Ala
180 185 190

Val Ala Lys Gly Ala Ala Lys Glu Ile Asn Leu Leu Ile Gly Thr Lys
195 200 205

Pro Val Met Lys Ala Ser Val Phe Ser Ser Asp Ser Lys Leu Ala Glu
210 215 220

Ser Asn
225